REMARKS

The amendment and these remarks are responsive to the Office Action dated February 13, 2004. Claims 6-20, 27 and 28 are pending. Claims 6-20 have been withdrawn. Claims 27 and 28 are rejected. Claims 27 and 28 are objected to. Claims 27 and 28 are amended. New claims 29 and 30 have been added.

In view of the foregoing amendments and the following remarks, applicants request reconsideration under 37 C.R.F. § 1.111 and allowance of the pending claims.

Claim Objections

Claims 27 and 28 are objected to as containing informalities. In particular, the action states that it is unclear if all the probes of claims 27 and 28 are listed in the alternative.

The applicants appreciate the careful review of the claims, and have amended claims 27 and 28 as suggested in the action. In particular, in claim 27, lines 25 and 26, and in claim 28, lines 10 and 11, the word "and" is replaced with the word "or." Additionally, in claim 27 a hyphen (-) is added between the numbers 12 and 15 in line 32.

Applicants suggest that claims 27 and 28 are clear and precise, and comply with all formal requirements, and therefore request the withdrawal of the objections to claims 27 and 28.

Claim Rejections under 35 USC §103

Claims 27 and 28 are rejected under 35 USC 103 (a) as being unpatentable over Kilpatrick (US Patent 6,168,917): 102(e) dated July 9, 1999) in view of Accession numbers

U22521 (Jan 1997), AF177911 (Sep 1999), AF136379 (Jun 2000), U55870 (May 1996) and Z78129 (Aug 1997) and further in view of Accession number E30248 (from JP 1999346799, published Dec 1999). In particular, the action states that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to develop primer pairs and nucleotide sequences to detect Enterovirus 71 and Coxsackievirus A16 and to package such sequences in kit format, particularly as the sequences of enterovirus 71 and coxsackievirus A16 were known in the art at the time of the invention, and as Kilpatrick specifically teaches primer pairs that detect specific serotypes. Applicants respectfully disagree.

As previously argued, the applicants assert that the cited references fail to suggest the discrete conserved portions of the enterovirus nucleotide sequences set out in claims 27 and 28, the utility of such conserved regions for simultaneous detection and differentiation of enterovirus, and the advantageous sensitivity of a kit including a nucleotide sequence selected from SEQ ID NOs: 9-15 attached to a solid substrate as set out in claims 27 and 28. As a showing of the unexpected and advantageous properties of kits, applicants previously provided an exemplary article in a peer-reviewed scientific journal (Shih et al. (*Journal of Virological Methods* 111, 55-60 (2003))).

The action states that the previous arguments with regard to the unexpected and improved sensitivity of the kits claims in claims 27 and 28 would be persuasive if filed in an appropriate affidavit or declaration. Applicants therefore take this opportunity to submit the Declaration of Shin-Hwan Wang submitted under 37 C.F.R. § 1.132 (enclosed).

As set out in the enclosed Declaration of Dr. Wang, the sensitivity of the claimed kit of the present invention is approximately 2,400-24,000 times more sensitive than that of

Kilpatrick. The sensitivity of the kits in claims 27 and 28 would not be considered obvious by people of ordinary skill in the art in view of the cited references. Although the Kilpatrick reference teaches a method to detect minority populations of enteroviruses in mixed serotype cultures, the Kilpatrick reference neither teaches nor suggests the unexpected sensitivity of the presently claimed kits. Accordingly, applicants assert that the *prima facie* obviousness of claims 27 and 28 is hereby rebutted, and that a rejection of those claims under 35 USC § 103 is inappropriate. Applicants therefore respectfully request the withdrawal of the rejection of claims 27 and 28.

Rejoinder of Process Claims

Applicants suggest that claims 27 and 28, as amended, are in condition for allowance. As set out at MPEP § 821.04, in applications that disclose a product and a process for making and/or using the product, where applicant elects claims directed to the product that are subsequently found allowable, withdrawn process claims which depend from or otherwise include all the limitations of the allowable product claim will be rejoined. Such process claims will be entered as a matter of right if the amendment is presented prior to final rejection or allowance

Applicants have added new process claims 29 and 30 that include all the limitations of the allowable product claims. Applicants assert that the added method claims should be rejoined in the application and are also in condition for allowance.

The above amendments and remarks are believed to address fully the Examiner's

rejections, and place the application in condition for allowance. A prompt indication of the same respectfully is requested. The Examiner is encouraged to telephone the undersigned if any issues remain that may be resolved by a telephonic interview.

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, Washington, D.C. 20231, on May 13, 2004.

George Painter
George Painter
Date of Signature: May 13, 2004

Respectfully submitted,
KOLISCH HARTWELL, P.C.

Anton E. Skaugset
Anton E. Skaugset
Customer No. 23581
Registration No. 38,617
of Attorneys for Applicants
520 S.W. Yamhill Street, Suite 200
Portland, Oregon 97204
Telephone: (503) 224-6655
Facsimile: (503) 295-6679

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Lee, et al.
Serial No:	09/724,678
Filing Date:	11/28/2000
Title:	"Methods for Detecting and Differentiating Enteroviruses and the Primers and Probes Therefor"
Examiner:	Jehanne Souaya Sitton
Art Unit:	1634

DECLARATION UNDER RULE 1.132

I, Shin-Hwan Wang, do hereby declare and say:

My home address is 2F, No.13, Hubin 1st Rd., Hsinchu City 300, Taiwan (R.O.C.).

I have a Ph.D. degree in Chemistry from National Tsing Hua University.

I have worked in biochip field for seven years. I have been a member of Taiwan Biochip Association for three years.

I have been employed by DR. Chip Biotechnology Incorporation for four years. I am currently the General Manager.

1. I am one of the inventors in the subject application and fully familiar with the subject matter of the application and the references relied upon by the Examiner in the prosecution of this application.

2. I provided experimentation to determine the sensitivity of the claimed kit in the present invention. The experimentation was related to a DNA microchip,

which was an embodiment of the currently claimed kits in this invention. The methods and materials described in the following experimentation are largely the same as the presently claimed invention including the design of the primers (SEQ ID NOs: 4, 5, 6 and 8) and the probes (SEQ ID NOs: 9 to 15)

3. Experimentation

3.1. Clinical specimens

One hundred clinical specimens (throat swabs) were tested for enterovirus 71 using the novel microchip. All of the specimens were from the Clinical Virology Laboratory of Chang Gung Memorial Hospital and were taken in 2000 and 2001. The specimens were collected from patients with clinical features of hand, foot and mouth disease, herpangina, aseptic meningitis, encephalitis or unspecified respiratory tract infection. All of the specimens were identified as positive for enteroviruses by viral culture and IFA using a pan-enterovirus antibody (Chemicon, Temecula, CA). Furthermore, 67 specimens were identified as positive for enterovirus 71, 23 as coxsackievirus A16, one as echovirus 30, five as echovirus 4 and four as coxsackievirus B4. A type-specific antibody (Chemicon) was used for serotype identification. Cell lines used for virus isolation and propagation were RD cells, MRC-5 cells and vero cells. A neutralization assay was conducted with RD cells following the standard procedure for typing enteroviruses (World Health Organization, 1988).

3.2. RNA isolation, cDNA synthesis, and PCR amplification

Viral RNA isolation and purification were carried out using a QIAmp Viral RNA Mini Kit (Qiagen GmbH, Germany) according to the manufacturer's directions. Briefly, 140 μl of specimen was added to 560 μl lysis buffer, and the

mixture was incubated at room temperature for 10 min. 560 μl of ethanol was then added to each sample, and the suspensions were subjected to the QIAmp spin column. After centrifuging at 6000 × g for 1 min, the column was washed with 500 μl of buffers AW1 and AW2, and RNA was eluted with 60 μl elution buffer. Ready-to Go™ RT-PCR Beads (Amersham Pharmacia Biotech, Inc, US) were used to amplify the viral RNA, and each bead was reconstituted with 19 μl of DEPC-treated water and 1 μl of random hexamer (2.5 μg/μl; Amersham Pharmacia Biotech). A template RNA (30μl) was immediately added to the appropriate tube for 30 min at 42 °C, and the enzyme was inactivated by increasing the reaction temperature to 90 °C for 5 min. 12.5 μl aliquots of the RT mixture were then used as templates for PCR. The amplification reaction mixtures are templates for PCR. The amplification reaction mixtures (25 μl) contained 1.5 U DNA polymerase (Promega, Madison, WI, USA) and EV Mix (11.5 μl; DR. Chip Biotech, HsinChu, Taiwan). The EV Mix contained two sets of primers (Table 1), and the 5' end of the reverse primers were biotinylated. A DNA template derived from human β-actin (GenBank accession No. m10277, position 2034-2346) and one set of actin primers (15 nucleotides of both ends) were also included in the EV Mix as the PCR internal control. A PTC-100™ Programmable Thermal Controller (MF Research, Inc.) thermal cycler was used to perform the following program: 94 °C for 4 min (1 cycle); 94 °C for 50 s, 55 °C for 50 s, and 72 °C for 50 s (40 cycles); and extension at 72 °C for 4 min.

Table 1

Oligonucleotide sequence of primers used for RT-PCR amplification and probes in the microchip

Primer	Sequences (5'→3')	Location	Genomic position
Evf5	AAGAGYCTATTGAGCTA	5'-NCR	423-439
Evr1	CACYGGATGGCCAATCCAA	5'-NCR	627-645
Evf7	GGITGGTRSTGGAARTTICC	VP2	1179-1198
Evr4	ARRTTIATCCAYTGRTGIGG	VP2	1485-1504
Probe			
Pan EV-1	TCCTCCGGCCCCTGAATGCGGCTA ATC	5'-NCR	448-535
Pan EV-2	TGTCGTAACGSGCAASTCYGYRGC GGAACCGAC	5'-NCR	514-546
Pan EV-3	TACTTTGGGTGTCCGTGTTTCHTTT TAT	5'-NCR	547-574
EV 71-1	CTTATAAGCAGACTCAACCCGGTG CTGATG	VP2	1390-1419
EV 71-2	TGGCATTCCAATATCACAATTAACA GTG	VP2	1453-1481
EV 71-3	TRCARCACCCGTACGTGCTYGATGCT GGSA	VP2	1429-1458
CA 16-1	CTCGGCACTATCGCAGGAGGGACC GGGAAT	VP2	1343-1373
CA 16-2	CCTACGCCACTACACAGCCTGGTC AGGTTG	VP2	1390-1419
Probe-P	GAGCGGGAAATCGTGCGCGACATCA AGGAG	Actin	2278-2307
Probe-H	ATGAAGCAYGTCAGGGCRTGGATACC TCG	VP1	3201-3229

3.2. Hybridization on the EV^{TM} chip

The amplification reaction mixtures contained two sets of primers (both 5'-noncoding region (NCR) and VP2 primers), and the RT-PCR products were hybridized together in the chip. On the other hand, specific oligonucleotides were immobilized at known locations on a polymer substrate to form an enterovirus 71-microchip (DR. EV^{TM} Chip; DR. Chip Biotech). Equal amounts of probes Panenterovirus-1, Panenterovirus-2, and Panenterovirus-3 were mixed together and then immobilized at the locations named "spot 5." Using the same method, the spot 6 locations contained the mixture of probes enterovirus 71-1, enterovirus 71-2 and enterovirus 71-3, and the spot 7 locations contained a mixture of probes coxsackievirus A 16-1 and coxsackievirus A 16-2. 8-μl aliquot

of the PCR product in a 1.5-ml tube was denatured at 94 °C for 5 min and chilled on ice for 2-3 min. Following denaturation, 392 μl of hybridization buffer (DR. Hyb buffer, ER. Chip; 6× SSC, 5× Denhardt's reagent, 0.5% SDS, 100 μg/ml salmon sperm DNA) was added, thoroughly mixed and placed into the reaction chamber within the DR. EV™ chip. The hybridization buffer also contained 15 nM of biotin-labeled oilgonucleotide that is complementary to hybridization positive control probe (probe-H) listed in Table 1. Hybridization control displays a blue color when the hybridization reaction is successful. The chamber was placed in an oven (DR. Hyb™ oven, DR. Chip) for 1 h at 50 °C. The hybridization solution was then discarded, and each chamber was washed five times with 500 μl of washing buffer (DR. chip, 0.1 M maleic acid, 0.15 M NaCl, pH 7.5). Next, the streptavidin alkaline phosphatase (Promega) was diluted in the blocking buffer (Roach Diagnostics GmbH, Mannheim, Germany) to a concentration of 0.5 μl/ml and added to each chip (400 μl/chip). The chips were then incubated for 30 min at room temperature, and after 30 min of incubation, they were washed five times with 500 μl of washing buffer, and 400 μl of the colorimetric substrate (Roche Diagnostics GmbH, Mannheim, Germany) was added to each chip. The colorimetric substrate was prepared by diluting NBT/BCIP stock solution (Roche) in a detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5) to a concentration of 20 μl per ml. After 10 min of incubation in the dark at room temperature, the chips were washed twice with 500 μl of washing buffer (DR. Chip) and air-dried.

3.3. Results

One hundred specimens from patients infected with enteroviruses were analyzed (Table 2), all of which had previously been confirmed as enterovirus

infection by viral culture. The enterovirus 71-microchip obtained positive results for 60 (89.6 %) of the 67 specimens identified as enterovirus 71 by viral culture. Meanwhile, of the 33 non-enterovirus 71 enteroviruses were also tested in this investigation, 3 (9.1 %) showed positive for enterovirus 71, including one case of coxsackievirus A16 and two of echovirus 4. Taking the traditional viral culture method as a reference, EV71-specimens that tested positive with the microchip but negative by the culture method were considered false positive results. Conversely, EV71-specimens that tested positive using the culture method but negative using the microchip were considered to by false negative results. The number of true positive, true negative, false positive and false negative results were 60, 30, 3 and 7, respectively. The novel microchip thus has a sensitivity of 89.6 % and specificity of 90.9 %.

Table 2

100 clinical specimens tested with EV71-microchip

Virus	Total number	Positive number
Enterovirus 71	67	60
Coxsackievirus A16	23	1
Echovirus 30	1	0
Echovirus 4	5	2
Coxsackievirus B4	4	0

The results of the experimentation show that the detection capability of a microchip is more sensitive than that of a PCR reaction. To determine how many viruses must be present in a specimen before they can be detected by the EV71-microchip, a series of various dilutions of viruses (strain BrCr) were added to a negative specimen. The virus titer was measured by plaque assay. Meanwhile, RT-PCR was performed with RNA samples isolated from each of

the serial dilutions. The amount of template RNA corresponding to 10^2-10^3 virions was needed to produce a visible specific amplicon on the agarose gel (data not shown). However, the EV71-microchip detects the amplicon derived from viral RNA corresponding to 1-10 virions.

4. US patent No. 6,168,917 (Kilpatrick et al.) teaches a method to detect an enterovirus in a sample, and 100 fg RNA is used as a template in a sample (see column 20, line 10). To compare the sensitivity between the current application and Kilpatrick's patent, we first estimate how many copy numbers of enterovirus genomes equals 100 fg RNA. 100 fg RNA is about 2,400 copy numbers calculated by the following equation.

The equation is $100\text{fg} \times 10^{-15}/\{(7500 \times 330)/6.022 \times 10^{23}\}$; then

$100\text{fg} \times 10^{-15} \times 6.022 \times 10^{23}/(7500 \times 330)$ is about 2,400, wherein 6.022×10^{23} is *Avogadro's number*, the genome of an enterovirus is about 7500 nt, and the average molecular weight of a nucleotide is 330 g/mole, so that 2,400 is the copy numbers of the enterovirus genome. When a practitioner uses the methods according to Kilpatrick's patent to detect enteroviruses in a sample, the templates in the sample must reach 2,400 copy numbers of the enterovirus genome. However, the currently claimed kits detect 1-10 virions in a sample (see above) and is more sensitive than US Patent No. 6,618,917.

5. Base on the foregoing description, my professional opinion is that Kilpatrick's patent neither teaches nor suggests the unexpected sensitivity of the presently claimed kits. Furthermore, Kilpatrick's patent neither discloses nor specifically demonstrates primer pairs and probes to Vp2. Accordingly, the presently claimed kits are not obvious to people skilled in the art in light of

US Patent No. 6,618,917.

6. I, Shin-Hwan Wang, the undersigned declarant, further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

Signed this 9th of April, 2004.

Shin-Hwan Wang, Ph.D.

Shin-Hwan Wang